

CLAIMS

1. A, recombinant fusion polypeptide comprising a first polypeptide peptide bonded to a second polypeptide, wherein said first and second polypeptides are found in nature as monomers of a multimeric protein and said first and second polypeptides are not fluorescent donor and
5 acceptor to each other, and wherein said recombinant fusion polypeptide is fluorescent when excited.
2. The recombinant fusion polypeptide of claim 1 wherein said first polypeptide and said second polypeptide are peptide bonded to each other via a linker sequence.
3. The recombinant fusion polypeptide of claim 2 wherein said linker sequence is from 5 to
10 50 amino acids long.
4. The recombinant fusion polypeptide of claim 1, further comprising a third polypeptide peptide bonded to said recombinant fusion polypeptide.
5. The recombinant fusion polypeptide of claim 4 wherein said third polypeptide is a member of a specific binding pair.
- 15 6. The recombinant fusion polypeptide of claim 4 wherein said third polypeptide is fused to the amino terminus of said first polypeptide.
7. The recombinant fusion polypeptide of claim 4 wherein said third polypeptide is fused to the carboxy terminus of said second polypeptide sequence.
8. The recombinant fusion polypeptide of claim 1 wherein each of said first and said second
20 polypeptide, independently, is a monomer of a multimeric protein selected from the group consisting of: *R. reniformis* GFP, *R. mulleri* GFP and *A. victoria* GFP.
9. The recombinant fusion polypeptide of claim 1 wherein both of said first and second polypeptides are monomers of a single multimeric protein selected from the group consisting of *R. reniformis* GFP, *R. mulleri* GFP and *A. victoria* GFP.

10. A polynucleotide encoding a recombinant fusion polypeptide comprising a first polypeptide peptide bonded to a second polypeptide, wherein said first and second polypeptides are found in nature as monomers of a multimeric protein, and wherein said recombinant fusion polypeptide is fluorescent when excited.
- 5 11. The polynucleotide of claim 10 wherein said first polypeptide and said second polypeptide are peptide bonded to each other via a linker sequence.
12. The polynucleotide of claim 11 wherein said linker sequence is from 5 to 50 amino acids long.
13. The polynucleotide of claim 10, wherein said polynucleotide further encodes a third
10 polypeptide peptide bonded to said recombinant fusion polypeptide.
14. The polynucleotide of claim 13 wherein said third polypeptide is a member of a specific binding pair.
15. The polynucleotide of claim 13 wherein said third polypeptide is fused to the amino terminus of said first polypeptide.
- 15 16. The polynucleotide of claim 13 wherein said third polypeptide is fused to the carboxy terminus of said second polypeptide.
17. The polynucleotide of claim 10 wherein each of said first and said second polypeptide, independently, is a monomer of a multimeric protein selected from the group consisting of *R. reniformis* GFP, *R. mulleri* GFP, and *A. victoria* GFP.
- 20 18. The polynucleotide of claim 10 wherein both of said first and second polypeptides are monomers of a single multimeric protein selected from the group consisting of *R. reniformis* GFP, *R. mulleri* GFP, *A. victoria* GFP.
19. A vector comprising the polynucleotide of claim 10.

20. A cell comprising the vector of claim 19.
21. The cell of claim 20, said cell being a bacterial cell.
22. The cell of claim 20, said cell being a eukaryotic cell.
23. The eukaryotic cell of claim 22, wherein said cell is a yeast cell, an insect cell, or a
5 mammalian cell.
24. A pair of polypeptides comprising a polypeptide labeled with a fluorescent dye and a recombinant fusion polypeptide of claim 1 wherein said fluorescent dye and said recombinant fusion polypeptide are fluorescent donor and acceptor to each other.
25. A pair of recombinant fusion polypeptides comprising a first fusion polypeptide as
10 claimed in claim 1 and a second fusion polypeptide as claimed in claim 1 wherein said first fusion polypeptide and said second fusion polypeptide are fluorescent donor and acceptor to each other.
26. The pair of recombinant fusion polypeptides of claim 25 wherein each of said first and second fusion polypeptides further comprises a third polypeptide, and wherein said third
15 polypeptide of said first fusion polypeptide comprises a sequence which is different from said third polypeptide of said second fusion polypeptide.
27. A method of producing a fluorescently labeled recombinant fusion polypeptide, said method comprising the steps of introducing a polynucleotide of claim 10 to a cell, and culturing said cell under conditions that permit the synthesis of said recombinant fusion polypeptide,
20 whereby said recombinant fusion polypeptide is produced.
28. A method of labeling a cell with a fluorescent recombinant fusion polypeptide, said method comprising the steps of:
- (a) introducing a polynucleotide of claim 10 to a cell; and

(b) culturing said cell under conditions that permit the synthesis of said recombinant fusion polypeptide, whereby said cell is labeled with said fluorescent recombinant fusion polypeptide.

29. The method of claim 26 wherein, in said introducing step (a), said polynucleotide
5 introduced to said cell further comprises a sequence encoding a third polypeptide fused in frame to the sequence encoding said recombinant fusion polypeptide.

30. A method of monitoring the interaction of two polypeptides of interest, said method comprising the steps of:

- (a) contacting a first polypeptide and a second polypeptide wherein:
- 10 (i) said first polypeptide is a recombinant fusion polypeptide of claim 4 wherein said third polypeptide is a first polypeptide of interest;
- (ii) said second polypeptide comprises a second polypeptide of interest and is fluorescently labeled; and
- (iii) the fluorophores comprised by said first and second polypeptides are
15 fluorescent donor and fluorescent acceptor to each other;

(b) exciting said donor fluorophore; and

(c) detecting fluorescent emission from said fluorescent acceptor, wherein said emission is indicative of the interaction of said first and said second polypeptides of interest.

31. The method of claim 30 wherein said second polypeptide comprises a fusion polypeptide
20 of claim 5, wherein said third polypeptide of said second fusion polypeptide is different from said third polypeptide of said first fusion polypeptide.

32. The method of claim 30 wherein said contacting step is performed in vitro.

33. The method of claim 30 wherein said contacting step is performed in a cell.

34. The method of claim 33 wherein said contacting comprises the step of introducing nucleic acid encoding said first and said second polypeptides to a cell.

35. A method of screening for a compound that modulates the interaction of a first and a second member of a specific binding pair, said method comprising the steps of:

5 (a) contacting a first polypeptide and a second polypeptide in the presence and absence of a candidate modulator wherein:

(i) said first polypeptide is a recombinant fusion polypeptide of claim 5, wherein said member of a specific binding pair is said first member of a specific binding pair;

10 (ii) said second polypeptide is fluorescently labeled and comprises said second member of a specific binding pair; and

(iii) the fluorophores comprised by said first and second polypeptides are fluorescent donor and, acceptor to each other;

(b) exciting said donor fluorophore; and

15 (c) detecting the fluorescence of said acceptor fluorophore, wherein emission of the spectrum characteristic of said fluorescent acceptor indicates the interaction of said first and said second members of said specific binding pair, and wherein a change in said interaction in the presence of said candidate modulator indicates that said candidate modulator modulates the interaction of the members of said specific binding pair.

20 36. The method of claim 35 wherein said second polypeptide is a recombinant fusion polypeptide of claim 5 and said member of a specific binding pair comprised by said second polypeptide is said second member of a specific binding pair.